Neuron
Previews

A Single Sensory Neuron Directs Both Attractive and Repulsive Odor Preferences

Ikue Mori[1,*]
[1]Group of Molecular Neurobiology, Division of Biological Science, Graduate School of Science, Nagoya University and CREST-JST, Nagoya, 464-8602, Japan
*Correspondence: m46920a@nucc.cc.nagoya-u.ac.jp
DOI 10.1016/j.neuron.2008.09.016

Animal behaviors are subjected to innate preferences, which are usually encoded by dedicated sensory neurons. In this issue of *Neuron*, Tsunozaki and colleagues show that one olfactory neuron in *Caenorhabditis elegans* produces two opposing preferences to an odor by regulating cGMP and PKC signaling.

Animals can recognize and respond appropriately to their surrounding environment by the relay of the neural processing. A particular stimulus is sensed by sensory neurons of the peripheral nervous system, the neural signal is integrated by interneurons of the central nervous system, and the result of neural computation is executed as a behavioral output. It has been a general understanding that innate preferences to major environmental cues such as smell and taste are encoded in respective sensory neurons. Given a complete anatomical connectivity map between the identifiable 302 neurons in the nervous system along with powerful genetics tools, *C. elegans* is an ideal animal model system to address at the molecular, cellular, and neural circuit levels the issue of how sensory neurons perceive environmental stimuli. Olfactory neurons are among the most characterized sensory neurons in *C. elegans* (Bargmann, 2006, for review). Wild-type animals show attractive behavioral responses to odorants sensed by two pairs of AWC and AWA sensory neurons, whereas they show repulsive responses to odorants sensed by three pairs of AWB, ASH, and ADL sensory neurons. These observations provide a good example for innate olfactory preferences that are encoded by dedicated sensory neurons.

In this issue of *Neuron*, Tsunozaki et al. (2008) reported exceptional aversive olfactory responses by AWC neuron. The two AWC neurons, designated AWC^{ON} and AWC^{OFF} neuron, respectively, are similar to each other but partly different in odor sensation. The AWC^{ON} neuron senses butanone, the AWC^{OFF} neuron senses 2,3-pentanedione, and both AWC^{ON} and AWC^{OFF} neurons sense benzaldehyde and isoamyl alcohol (Troemel et al., 1999; Wes and Bargmann, 2001). Tsunozaki et al. (2008) attempted to isolate mutants defective in attractive chemotaxis to butanone. A genetic screen isolated a novel loss-of-function mutant in the *gcy-28* gene, which encodes several receptor-type guanylate cyclases, and showed that they are not merely unresponsive to butanone but exhibit repulsive chemotaxis. Similar to wild-type animals, *gcy-28* mutants were attracted to odors sensed by AWA neurons, suggesting that chemotaxis to AWA-sensed odors is normal. Although *gcy-28* mutants were less attracted to other odors sensed by AWC neurons, they never showed repulsive chemotaxis to those AWC-sensed odors, suggesting that butanone sensation induces repulsive olfactory behavior in *gcy-28* mutants.

Butanone is sensed by AWC^{ON} neurons to direct attractive chemotaxis in wild-type animals. Similarly, repulsion is usually directed by so-called avoidance neurons (AWB, ASH, and ADL). Tsunozaki et al. (2008) addressed whether repulsive responses to butanone in *gcy-28* mutants are caused by impaired function of AWC^{ON} neuron or avoidance neurons. *nsy-5 gcy-28* double mutants with two AWC^{OFF} neurons did not show any response to butanone, and *gcy-28*; *nsy-1* double mutants with two AWC^{ON} neurons showed enhanced repulsive response to butanone. These and other results suggest that repulsive chemotaxis to butanone in *gcy-28* mutants is ascribed to AWC^{ON} neurons. Consistent with the importance of *gcy-28* mutation in AWC^{ON} neurons for butanone repulsion, mutations affecting the function of avoidance neurons did not cause repulsive responses to butanone. Remarkably, *gcy-28*; *nsy-1* double mutants with two AWC^{ON} neurons reversed benzaldehyde chemotaxis from attractive to repulsive. Thus, the lack or reduction of GCY-28 receptor-type guanylate cyclases in AWC^{ON} neurons directs general repulsive chemotaxis behaviors in accordance with odor sensing in AWC^{ON} neurons.

To clarify the behavioral basis of butanone repulsion, Tsunozaki et al. (2008) conducted behavioral analysis based on the biased random-walk model, which is now commonly used for analysis of other behaviors in *C. elegans* (Pierce-Shimomura et al., 1999). Turn frequency was used as a measure of change in direction of movement. Wild-type animals increased their turning frequency when migrating down the butanone gradient and decreased their turning frequency when migrating up the gradient. By contrast, *gcy-28* mutants increased their turning frequency when migrating up the butanone gradient and decreased their turning frequency when migrating down the gradient. Thus, *gcy-28* mutants exhibit the reversed turning bias to butanone. The turning bias on the butanone gradient was abolished when AWC^{ON} neurons were killed by a laser microbeam in both wild-type and *gcy-28* animals, suggesting that AWC^{ON} neurons are required for butanone repulsion.

gcy-28 genes appear to produce several transcripts due to alternative splicing. Of cDNAs derived from several splicing variants, expression of one cDNA isoform in AWC^{ON} neurons of *gcy-28* mutants gave rise to full restoration of butanone chemotaxis, suggesting that *gcy-28* acts cell autonomously in AWC^{ON} neurons. This cDNA isoform possessing full rescuing activity was fused to the AWC^{ON}-selective

promoter and the *gfp* gene to visualize the intracellular site of action. Tsunozaki et al. (2008) observed that functional GFP-tagged GCY-28 protein was profoundly localized to AWC axon, where neural signals should be conveyed to other neurons by synaptic connections. Axonal localization of GCY-28 is quite remarkable, since other known receptor-type guanylate cyclases such as ODR-1 and DAF-11 are localized to sensory cilia, which are the site for primary sensory transduction. To investigate directly whether GCY-28 mediates primary sensory transduction, Tsunozaki et al. (2008) measured odor-evoked calcium transient in AWC^{ON} neurons of *gcy-28* mutants by expressing the genetically encoded calcium indicator G-CaMP. As recently reported by the same group (Chalasani et al., 2007), the removal from odor immediately induced large increments of intracellular calcium levels in AWC^{ON} neurons of wild-type animals. Similar change in odor-evoked intracellular calcium levels was detected in *gcy-28* mutants. The addition of odor did not affect the calcium levels in AWC^{ON} neurons of both wild-type and *gcy-28* animals. These results suggest that GCY-28 is not directly associated with primary olfactory transduction in AWC^{ON} neurons.

If GCY-28 is not necessary in odor-dependent primary sensory transduction, what is the role of GCY-28 on chemotaxis to AWC^{ON}-sensed odors? *pkc-1/ttx-4* gene encodes a novel protein kinase C-epsilon/eta, which is activated by diacylglycerol (DAG) and is required for various sensory behaviors including chemotaxis to AWC-sensed odors (Okochi et al., 2005). PKC-1 is also reported to regulate synaptic neuropeptide secretion at the neuromuscular junction (Sieburth et al., 2007). The localization of GCY-28 to AWC axons is consistent with the possibility that like PKC-1, GCY-28 would function at AWC synaptic release or secretion to direct chemotaxis to AWC^{ON}-sensed odors. This possibility is further supported by the following results (Tsunozaki et al., 2008): *pkc-1* single mutants and *gcy-28; pkc-1* double mutants showed similar repulsive butanone chemotaxis to that of *gcy-28* single mutants, suggesting that the two genes have a similar or related function. Calcium imaging revealed normal butanone-evoked calcium transient in AWC^{ON} neurons of *pkc-1* mutants, suggesting that like GCY-28, PKC-1 is not involved in primary sensory transduction. Further, activation of PKC-1 by expressing constitutively active PKC-1 in AWC fully rescued repulsive butanone chemotaxis of *gcy-28* mutants. A diacylglycerol kinase (DGK-1) that hydrolyzes DAG to phosphatidic acid (PA) is known to affect DAG signaling and cholinergic neurotransmission at the neuromuscular junction (Lackner et al., 1999; Nurrish et al., 1999). In contrast to PKC-1, the lack of DGK-1 by *dgk-1* mutation almost completely suppressed repulsive butanone chemotaxis of *gcy-28* mutants: *gcy-28; dgk-1* double mutants showed attractive butanone chemotaxis. Likewise, treatment of *gcy-28* mutants with PMA, a pharmacological agonist of DAG signaling, suppressed *gcy-28* chemotaxis defect. Altogether, these results suggest that increased DAG signaling suppresses the chemotaxis deficit of *gcy-28* mutants, possibly through synaptic releases.

C. elegans is capable of modifying its behavior after conditioning. For example, odor conditioning in the absence of food causes adaptation, or reduced chemotaxis to odor (Colbert and Bargmann, 1995), and odor conditioning in the presence of food causes sensitization, or enhanced chemotaxis to odor (Torayama et al., 2007). Based on the hypothesis that these context-dependent behaviors affect competition between attractive and repulsive activities of AWC, Tsunozaki et al. (2008) tested chemotaxis to butanone sensed by AWC^{ON} neuron in wild-type and *gcy-28* mutants after butanone conditioning. Notably, wild-type animals showed butanone repulsion after long-term exposure to butanone (2 hr). *gcy-28* mutants did not show enhanced butanone repulsion after butanone conditioning. Thus, the behavior of wild-type animals after AWC^{ON}-sensed butanone conditioning is similar to the behavior of naive *gcy-28* mutants showing repulsive chemotaxis to AWC^{ON}-sensed butanone. These results suggest that naive *gcy-28* mutants exhibiting repulsive butanone chemotaxis are a reflection of wild-type animals in a butanone-adapted behavioral state, thereby further implicating the essential mechanism controlling behavioral reversals.

In sum, the main contribution of Tsunozaki et al. (2008) is to show that a single sensory neuron can possess the capacity to rapidly reverse behavioral preference to an environmental stimulus. This neuron property is unprecedented, as other sensory neurons analyzed in *C. elegans* have been shown to direct attraction or repulsion, but not both. A gustatory neuron pair driving attraction, ASER and ASEL, may be functionally similar to the olfactory neuron pair, AWC^{ON} and AWC^{OFF}, since both systems show between-cell behavioral asymmetries (Hobert et al., 2002; Suzuki et al., 2008). However, Tsunozaki et al. (2008) now show that within-cell behavioral asymmetry can also occur, thus increasing the complexity and hence the adaptability of this sensory circuit in response to environmental cues. Future work may address the molecular mechanism by which the cGMP and PKC pathways in AWC^{ON} produces the switch in behavioral preference, define how developmental and environmental factors guide the role of AWC^{ON} in the *C. elegans* behavioral repertoire, and perhaps reveal further examples of single cell control modules in other sensory circuits.

REFERENCES

Bargmann, C.I. (2006). In WormBook, The *C. elegans* Research Community, ed. 10.1895/wormbook.1.123.1, http://www.wormbook.org.

Chalasani, S.H., Chronis, N., Tsunozaki, M., Gray, J.M., Ramot, D., Goodman, M.B., and Bargmann, C.I. (2007). Nature *450*, 63–70.

Colbert, H.A., and Bargmann, C.I. (1995). Neuron *14*, 803–812.

Hobert, O., Johnston, R.J., Jr., and Chang, S. (2002). Nat. Rev. Neurosci. *3*, 629–640.

Lackner, M.R., Nurrish, S.J., and Kaplan, J.M. (1999). Neuron *24*, 335–346.

Nurrish, S., Segalat, L., and Kaplan, J.M. (1999). Neuron *24*, 231–242.

Okochi, Y., Kimura, K.D., Ohta, A., and Mori, I. (2005). EMBO J. *24*, 2127–2137.

Pierce-Shimomura, J.T., Morse, T.M., and Lockery, S.R. (1999). J. Neurosci. *19*, 9557–9569.

Sieburth, D., Madison, J.M., and Kaplan, J.M. (2007). Nat. Neurosci. *10*, 49–57.

Suzuki, H., Thiele, T.R., Faumont, S., Ezcurra, M., Lockery, S.R., and Schafer, W.R. (2008). Nature *454*, 114–117.

Torayama, I., Ishihara, T., and Katsura, I. (2007). J. Neurosci. *27*, 741–750.

Troemel, E.R., Sagasti, A., and Bargmann, C.I. (1999). Cell *99*, 387–398.

Tsunozaki, M., Chalasani, S.H., and Bargmann, C.I. (2008). Neuron *59*, this issue, 959–971.

Wes, P.D., and Bargmann, C.I. (2001). Nature *410*, 698–701.